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☐ 1: J Biomol NMR. 2003 Nov;27(3):193-203.

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**<sup>1</sup>H(C) and (<sup>1</sup>H(N) total NOE correlations in a single 3D NMR experiment. (<sup>15</sup>N and (<sup>13</sup>C time-sharing in t(1) and t(2) dimensions for simultaneous data acquisition.**

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Simultaneous data acquisition in time-sharing (TS) multi-dimensional NMR experiments has been shown an effective means to reduce experimental time, and thus to accelerate structure determination of proteins. This has been accomplished by spin evolution time-sharing of the X and Y heteronuclei, such as (<sup>15</sup>N and (<sup>13</sup>C, in one of the time dimensions. In this work, we report a new 3D TS experiment, which allows simultaneous (<sup>13</sup>C and (<sup>15</sup>N spin labeling coherence in both t(1) and t(2) dimensions to give four NOESY spectra in a single 3D experiment. These spectra represent total NOE correlations between (<sup>1</sup>H(N) and (<sup>1</sup>H(C) resonances. This strategy of double time-sharing (2TS) results in an overall four-fold reduction in experimental time compared with its conventional counterpart. This 3D 2TS CN-CN-H HSQC-NOESY-HSQC pulse sequence also demonstrates improvements in water suppression, (<sup>15</sup>N spectral resolution and sensitivity, which were developed based on 2D TS CN-H HSQC and 3D TS H-CN-H NOESY-HSQC experiments. Combining the 3D TS and the 3D 2TS NOESY experiments, NOE assignment ambiguities and errors are considerably reduced. These results will be useful for rapid protein structure determination to complement the effort of discerning the functions of diverse genomic proteins.

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